REPORT OF THE HOSPITAL

APRIL 16, 1921

With the growth of the hospital in age and diversity of activities has come a corresponding growth of the staff in training and experience. Whereas during the first years of the hospital's existence most of the members of the staff consisted of men of relatively small experience and training in scientific work, now the hospital staff includes several men of wide reputation and scientific ability and capable of carrying on and directing scientific work. This has resulted in a greater or lesser separation of the work of the hospital into several divisions, each under the direction of one man, with a corresponding division of responsibility.

From the nature of the material which is studied it is obvious that this division of activities can never be so complete in the hospital as that which is possible in a purely laboratory organization. However, by maintaining as close cooperation as possible between the various divisions it is hoped that no loss in effectiveness has resulted or will result from this change in organization, but instead, that a great increase in individual accomplishment will follow.

As a result of this modification in the plan of work it has become increasingly difficult to present a report on the progress of the investigation of the various diseases which are being studied which will adequately describe the activities of the hospital. Consequently the hospital report now consists more of a report of the individual workers rather than, as formerly, of a

report of the work in the hospital as a whole.

The patients admitted for study have consisted of persons suffering from various forms of acute respiratory disease, acute rheumatic fever, measles, nephritis, or heart disease.

The study of respiratory disease has been directed along somewhat different lines than those in which the hospital has been engaged in the past. It is believed that the effectiveness of serum treatment in cases of pneumonia due to Type I pneumococcus has now been well demonstrated and the technique of treatment has been sufficiently well developed that it may be effectively employed in other hospitals. The manufacture of the serum also has been undertaken by others so that it has not seemed advisable to employ the resources of the hospital to any great extent in further study along this line. The study of the question of effective treatment of pneumonia, however, has not been discontinued since there are many cases of other types for which at present there is no effective treatment known. Our experience so far has not indicated that it will be possible to make any modification in the method of serum treatment which is employed in Type I cases to make it applicable to these other forms. Attempts are, therefore, being made through more fundamental studies to obtain new lines of approach to the problem of specific treatment in these other cases. In the meantime, it has been thought advisable to study more fully the functional abnormalities present in patients suffering from pneumonia

with the possibility that improvements in treatment may be devised, even in the absence of any form of specific treatment which acts directly upon the bacteria or their products. The progress made along these lines is described under the work of Dr Binger and Dr Stadie. The following report by Dr Avery includes a description of work accomplished and in progress relating to the bacteriology of respiratory infections.

Dr Avery.

Studies on Enzymes of Pneumococcus.

In conjunction with Dr Cullen the study of the biochemistry of the intracellular substances of pneumococcus has been continued; during the past six months, however, this study has been unavoidably interrupted because of demands made on Dr Cullen's time by the work of his own department form which his services could be spared only at odd times. In the November issue of the Journal of Experimental Medicine there appeared three papers by Dr Avery and Dr Cullen covering the studies on the enzymes of pneumococcus thus far completed. These deal with the proteolytic, lipolytic and carbohydrate-splitting enzymes and make clear the fact that, by means of sterile solutions and extracts of pneumococci, it is possible to correlate many of the functional activities of the living cell with the enzymotic processes of the intracellular substances removed from the growing organism.

Further study has demonstrated still other enzymotic activities of these pneumococcal substances. These specific intracellular agents manifest their activity on various substrates.

For instance, in the presence of blood, active solutions of pneumococci are strongly hemolytic and under proper conditions are capable of transforming hemoglobin into methemoglobin. Knowledge of the nature of these endohemotoxins may prove significant in the interpretation of certain blood changes clinically associated with pneumococcal infection in man. Again in the presence of bacterial substrates consisting of heat-killed pneumococci, these active enzyme-containing solutions cause complete and prompt lysis of the cell bodies; this bacteriolytic enzyme is specific in that its dissolving action is exerted only on pneumococci and not on hemolytic streptococci or staphylococci. On green-producing streptococci, which may be more closely related to pneumococcus than either of the other organisms, the bacteriolytic enzyme of pneumococcus exerts a lytic action, which, however, is never so active or so complete as in the case of the homologous organism itself.

In connection with the investigation of these intracellular enzymes, a study is being made of autolysis of pneumococci. It has been found, when washed bacterial cells from actively growing cultures are suspended in phosphate solutions, that the extent and rapidity of self-dissolution of the organisms is dependent upon the hydrogen ion concentration of the mixture. Autolysis is most rapid and complete over the zone pH 6.5 to 7.5; within this range lie the optima of the protease, lipase, and invertase of pneumococcus. In fact within this zone all types of enzymes of pneumococcus thus far described can act simultaneously. This fact recalls the observation of Dernby that autolysis of animal tissue

proceeds furthest at a hydrogen ion concentration where the various enzymes can function simultaneously.

In the course of this study it was observed that if the bacterial suspensions were autoclaved following autolysis, precipitation occurred at a definite reaction. Furthermore, this acid zone of heat precipitation was found to be different for different types of pneumococci and likewise different from the point of acid agglutination of the various types as determined by Gillespie. These observations suggested the possibility that this reaction might in some way be connected with differences in the isoelectric point of the cell protein. This phenomenon is now being investigated by the cataphoresis method.

Studies on Bile Solubility of Pneumococcus.

As far as is known for bacteria, solubility in bile is peculiar to pneumococcus alone. However, this property is possessed also by certain spirochaetes and trypanosomes. The fact that microorganisms so widely remote biologically should have this peculiarity in common, led earlier investigators to attempt the use in pneumococcal infections of chemotherapeutic agents of reputed value in the treatment of diseases due to organisms of the other groups. With the hope that a fuller knowledge of the mechanism of the bile reaction might lead to a better understanding of the action and effect of chemical and physical agents on the bacterial cell, study of the bile solubility of pneumococcus has been undertaken by Dr Avery.

Heating pneumococcus to a temperature above the thermal

death-point (52°C.), or subjecting the cells to an acidity equivalent to or greater than the acid death-point (pH 5.) renders them insoluble in bile. If the organisms are exposed to the action of carbolic acid or saturated with Gram's iodin solution and then washed free of these reagents, they are no longer soluble. The effect on bile solubility of varying the concentration of salt and of varying the hydrogen ion concentration of the fluid in which the organisms are suspended is being studied. The maximum solubility of the bacterial cell at any given concentration of salt from M/2 to M/320 is at pH 6., while on the acid side of this point, bile solubility decreases as the salt concentration increases, and at reactions more alkaline than pH 6., the greater the salt concentration the greater is the solubility of the cell. The explanation of these reactions is being sought at present.

Bacteria adsorb antibody and after specific adsorption, exhibit certain phenomena indicative of surface, if not of intracellular change. Specifically sensitized pneumococci are viable, agglutinated and rendered phagocytable; are they still bile soluble or is it possible that the cell membrane has become so altered as to be resistant to the action of bile? Pneumococci were sensitized in specific antipneumococcus serum, the agglutinated organisms thoroughly washed in repeated changes of salt solutions, the clumps of bacteria broken up, and the cells resuspended and tested for bile solubility. As control, pneumococci of the same type were exposed to heterologous immune serum and treated in the manner described. The specifically sensitized bacteria were bile insoluble, while

these similarly treated with non-specific serum were completely dissolved in bile.

As a large number of patients with lobar pneumonia give a history of some preceding infection of the respiratory tract, a study of the bacterial flora of the upper respiratory tract in health and in common colds has been started by Dr Ernest Stillman. Special attention has been paid to the bacterial flora of the nose where but few organisms, mostly various kinds of staphylococci, are found. The work on the biological classification of Bacillus influenzae has been confirmed and a study made of the distribution of these biological types. The incidence of Bacillus influenzae in about 100 normal throats this winter was 25 per cent. Of 11 cases of coryza and laryngitis studied, Bacillus influenzae was recovered in 5 cases. From 29 cases of lobar pneumonia Bacillus influenzae was recovered in 25 cases, or 87 per cent. From 2 cases of lobar pneumonia and from one case of laryngitis, meningococci were recovered from throat cultures. In 2 of these cases, the cultures were almost pure.

Studies concerning growth accessory substances in bacterial nutrition.

These studies are being carried on in conjunction with Dr Thjotta of Christiania, Director of the Laboratories of the Norwegian Army, who joined the hospital staff last November. Because of previous observations on the occurrence and growth of a mucoid strain of B. paratyphosus, Dr Thjotta became interested in the growth of mucoid bacilli in general and the transformation of

non-mucoid organisms into the mucoid variety. He began his work here with an attempt to grow different microorganisms, B. paratyphosus and pneumococci, in broth containing the mucus produced by a variety of mucoid bacilli. During this work he noted that Bacillus influenzae grew when inoculated into blood-free broth containing the mucoid material of Bacillus Friedländer. Considerable quantities of the mucoid substance were collected from growth of a mucus-producing strain of Bacillus Friedländer and prepared by suspending it in salt solution and heating it one hour at 60°C. This sterile substance, when added to broth in varying amounts was found to support life of Bacillus influenzae without the addition of blood or blood derivatives. Subsequently Dr Thjotta found a similar growth-promoting action in extracts of mucoid material obtained by boiling suspensions of Bacillus Friedländer and even of non-mucoid organisms such as Bacillus proteus. It is evident then that Bacillus influenzae can grow profusely in hemoglobin-free media consisting only of plain broth enriched by emulsions or extracts of mucoid bacilli or bacterium proteus.

Furthermore, it was found that these emulsions and extracts can be boiled for ten minutes and filtered through Berkefeld candles without losing their growth-inducing property. It was thought not unlikely that the growth-stimulating effect of these bacterial extracts might be due to substances of the same nature as the so-called vitamines. To test this assumption, similar extracts were prepared by Dr Thjotta and Dr Avery from yeast cells which are known to be rich in growth accessory substances. These extracts, even in minute amounts were found capable of promoting growth. Extraction of the growth

accessory substances from another source, namely, green vegetables, was tried; extracts of fresh tomatoes, green peas and string beans were found remarkably active in stimulating growth. These active yeast or vegetable extracts when added to broth greatly accelerate growth of organisms such as Bacillus influenzae and pneumococci, so that within five hours abundant growth is evident. In the case of pneumococci, a seeding too minute to initiate growth in plain broth alone will amply suffice to induce abundant growth in the same medium if a small amount of extract containing these growth accessory substances is added. In the case of Bacillus influenzae, luxuriant growth occurs in plain broth containing yeast extract when seeded from blood media, while under identical conditions, no growth takes place in the same broth to which these extracts have not been added. The addition of these growth accessory substances in concentration as small as 1:1000 suffices to stimulate growth under these conditions. However, for reasons to be discussed later, subsequent cultivation fails in broth medium containing only yeast or vegetable extracts.

While the nature of these growth accessory substances is not known, they are presumably analogous to the so-called vitamines. It has been found that they resist boiling for at least ten minutes, that they are destroyed by autoclaving, that they are extractable from fresh vegetables and from growing bacterial and yeast cells, that they are water soluble, that they pass a Berkefeld filter, and that extracts of these substances contain but little nitrogen,- about 0.116 per cent. Whatever their nature, it appears that these growth accessory substances are important factors in bacterial nutrition.

In the application of this principle to bacterial nutrition, particular attention has been thus far given to the nutritional requirements of Bacillus influenzae, since this organism belongs to a peculiar group of bacteria which have been heretofore considered obligate hemophiles. Closer analysis of the growth requirements of these organisms, however, has shown that the so-called hemophilic property has been based on a lack of knowledge of the actual nutritional needs. It was soon discovered that whereas Bacillus influenzae would grow luxuriantly when transplanted from blood medium to plain broth containing yeast extract, cultivation could not be continued in yeast broth alone more than one or two transfers. This suggested that possibly some other substance was carried over from the original blood culture in an amount sufficient to supplement the yeast broth and that growth failed in succeeding cultures because this substance was either exhausted by growth or lost by dilution on subsequent transfers. For purposes of discussion this substance may be referred to as the X factor and the vitamine-like substance in the extracts, as the V factor. Neither of these two factors by itself can sustain growth of Bacillus influenzae. Evidently both are essential to growth and both are supplied in blood used in the cultivation of Bacillus influenzae. As previously pointed out, the V factor is destroyed by autoclaving. If, therefore, blood medium is autoclaved, it should no longer be able to support growth of Bacillus influenzae. This is actually the case. If, however, the X factor has not been destroyed by heating, then this same medium should be reactivated by the addition of fresh yeast extract. This also is the case. The growth accessory substance (the

V factor) which is destroyed by autoclaving blood, can be supplied from another source, such as yeast; and this substance is capable of reactivating a medium in which, as a result of heating, the X factor alone remains. Moreover, just as the V factor can be supplied by the addition of extracts of living cells, so the X factor is found to be contained in hemoglobin-free serum and ascitic fluid. Serum broth containing yeast extract furnishes all the nutritive requirements for growth of Bacillus influenzae, containing as it does the requisite X and V factors. Search is being made for the X substance in material other than body tissue. That it is not hemoglobin itself, however, that contains this essential substance, is shown by the fact that pure crystalline hemoglobin when added to broth fails to support growth of Bacillus influenzae unless yeast or its equivalent in the V substance is also present. This fact indicates that hemoglobin contains some of the X factor, but is in itself not capable of supporting growth. Further, since substances other than hemoglobin have been found to furnish this X substance in greater abundance, it is evident that hemoglobin as such is not requisite to growth and that Bacillus influenzae is not a strictly hemoglobinophilic organism. This fact, however, is of less importance than the principle in bacterial nutrition which is involved in its demonstration. Further studies are planned to determine the importance of this principle in the cultivation of other species of bacteria. Similar analyses of the nutritional requirements of organisms now cultivated with difficulty may yield a fuller understanding of the optimal conditions for growth. From analogy with animal nutrition, it seems not unreasonable to suppose that

growth deficiency in the cultivation of bacteria may be overcome by the addition to culture media of the appropriate growth accessory substances.

Dr Blake

In association with Dr Trask the following studies have been made concerning measles:

1. Infectivity of the blood in experimental measles.

A series of experiments dealing with the infectivity of the blood in experimental measles in monkeys has been carried out. It has been shown that the disease may be readily carried through a series of animal passages by intravenous inoculation of whole blood from infected monkeys withdrawn during the prodromal and early eruptive stages. The infection has thus been carried through twelve passages. By this method of passage it has been found, however, that the virus gradually becomes attenuated until it is eventually no longer capable of causing a general infection. It has furthermore been shown that the virus is present in the plasma but not in the blood corpuscles, that it is present in as small amounts as 0.1 to 1 cc. of blood, and that it is capable of inducing a general infection by subcutaneous inoculation, provided it is so inoculated before it has been attenuated by animal passage.

2. Resistance of the virus to glycerol.

It has been shown that the virus remains viable and retains its infectivity for at least 2 months when preserved in 50 per cent glycerol in the icebox. Under these conditions it appears to become somewhat attenuated, though the data on this point are not as yet

sufficiently complete to make a definite statement.

3. Effect of intracutaneous injection of virus attenuated (?) by animal passage or glycerolization.

It has been found that monkeys inoculated intracutaneously with 0.1 to 0.5 cc. of fresh or glycerolated plasma containing an attenuated virus, exhibit a local reaction but no evidence of a general infection. The local reaction consists of a definite cutaneous edema of 12 to 48 hours duration which may or may not be followed in 2 or 3 days by a local exanthem in the neighborhood of the site of injection. A considerable number of monkeys have been thus injected to study the character of the local reaction. These monkeys are being kept for subsequent immunity studies which it is planned to begin soon.

4. Acquired immunity following experimental measles.

Six monkeys which had previously recovered from an attack of experimental measles have been reinoculated, in one instance with measles virus of homologous source, in five instances with virus of heterologous source. None of the six showed any evidence of infection while the control normal animals inoculated at the same time with equivalent amounts of the same material came down with experimental measles.

5. Continued attempts to cultivate the virus have not yet yielded positive results.

6. Attempted inoculation of rabbits with the virus of measles, by intravenous, subcutaneous and intratesticular routes have not been definitely successful, though yielding some suggestive results which warrant further study.

Dr Swift

With the assistance of Dr Boots and Dr Miller the following studies concerning acute rheumatic fever have been made or are in progress:

Ten patients who were considered to have rheumatic fever at the time of admission have been studied in the past six months; subsequently two of them have proven to have other forms of arthritis: one, a gonorrheal arthritis, the other a destructive arthritis. Of the eight patients in whom the diagnosis of rheumatic fever has been made, only two have had a simple polyarthritis without complication; the others have had distinct cardiac involvement. From pathological studies of the hearts of patients dying from this disease it is becoming more and more evident that a specific form of acute interstitial myocarditis is present in most fatal cases.. While in former times the chief emphasis was placed upon the occurrence of endocarditis in rheumatic fever, today we are making an effort to determine the presence of myocarditis during the course of the disease. In collaboration with Dr Cohn our patients are being studied with this point in view. Daily electrocardiograms are made of each patient during the acute stages of the disease, and at longer intervals at later stages; at the same time careful bedside studies are made to determine the relation of abnormalities in the electrocardiograms with signs of cardiac complications. While these studies are still only in the preliminary stage, it is evident that in many patients there are distinct symptoms and signs of myocardial involvement and at the same time evidence in electrocardiograms that the myocardium is acting in an abnormal

manner. Prolongation of the conduction time is one of the chief abnormalities noted; two of our patients have had transitory attacks of auricular fibrillation.

The action of the salicylates in rheumatic fever is not yet definitely determined: whether they act merely as antipyretics and analgesics, or whether they have a definite specific action upon the arthritis, are still moot points. Careful daily charts are being kept showing the development of each individual feature of inflammation in each joint and the disappearance of each of these signs and symptoms after the patient is fully under the influence of salicylates. No records of this type are available in the literature, and it is hoped from a study of patients in this manner to be able finally to throw some light upon this subject. Another clinical study continued from last year is the toxic action of salicylates upon the kidneys. Hanzlik and his coworkers in Cleveland have reported that most of their patients shortly after receiving toxic doses of the salicylates have edema and other evidence of disturbed renal function. Our studies in the past two years have failed to confirm these findings: Only when patients are severely intoxicated have they shown the symptoms that the above mentioned workers claim to have noted in most of their patients.

Blood and joint fluid cultures in all of our patients this year have failed to reveal the presence of streptococci or other bacteria. At present we are concentrating our attention upon cultures of the joint exudate with Noguchi's anaerobic methods. No definite results have been attained; grossly, some of the inoculated tubes have

appeared different than controls, but microscopic examinations have failed to reveal any definite bodies.

Our efforts to reproduce the disease in animals have been continued by the injection of rabbits, guinea pigs and monkeys with blood and exudates from patients. Four rabbits and two guinea pigs have shown a non-arthritis; all but one of these animals had been inoculated with material from other animals of the same species that had received the blood of rheumatic fever patients. It has, however, been impossible to induce arthritis in subsequently inoculated animals. So far, therefore, the clinical picture of polyarthritis rheumatica has not been reproduced experimentally. Histological studies are being made of the hearts of all of the inoculated animals; many of these show focal myocardial lesions the nature of which still awaits elucidation.

Dr Boots has carried out an extensive study upon the antibacterial properties of sodium salicylate in vitro and in vivo, and has compared these properties against streptococci of the non-hemolytic and green forming varieties with similar action against pneumococci and hemolytic streptococci. He has shown that sodium salicylate in concentrations five to ten times greater than it can exist in the body has practically no bactericidal action; not until a concentration of one per cent is reached is there any demonstrable inhibitory effect of the drug in fluid media. A theoretic dissociation curve of salicylic acid has been plotted, from which it is evident that salicylate in a medium of the reaction of the body must all be combined with base in the form of a salt; only at a pH of 5.

or less is there any free salicylic acid; at a pH of 4., less than ten per cent of the salicyl exists as free salicylic acid. It is obvious that this degree of acidity does not exist in the blood or inflammatory exudates of patients with rheumatic fever. It has been determined directly that the joint exudates of our patients is at a reaction of about pH 7.4; in one patient with Staphylococcus aureus arthritis the exudate had a reaction of pH 6.9. This evidence definitely controverts the long held opinion that the action of the salicyl in the arthritis of rheumatic fever was brought about by the acid reaction of the joint exudate in this disease; in this supposed acid exudate salicylic acid was supposed to be set free. It has been shown that salicylic acid at a reaction of pH 3.1 and 3.7 has little if any more bactericidal action than HCl of a similar hydrogen ion concentration. Staphylococci are more resistant to the salicylates than are the green streptococci; green streptococci, hemolytic streptococci and pneumococci are all about equally susceptible to the bactericidal action of the drug in vitro.

The blood of patients who have been given salicylates to the point of intoxication and that of rabbits receiving comparable doses has been tested for bactericidal properties: This blood was no more antibacterial than the blood of the same individual or animal before receiving salicylates, nor than that of normal controls. It seems evident from these studies that if we accept the green streptococcus as the etiologic agent in rheumatic fever - an opinion not at all proven - the beneficial effects of sodium salicylate must be exerted in some other way than against the bacteria.

Dr Van Slyke

Dr Cullen has been engaged in perfecting a simple colorimetric method for determination of hydrogen ion concentration in the blood plasma. Certain slight errors attend the use of indicators in such a solution as plasma. The proteins affect the behavior of the indicator, and to a slight extent, the salts also. Furthermore the pH undergoes an actual increase when the temperature of the plasma is changed from 38° to room temperature. Our purpose has been to devise a colorimetric technique which would yield constant results, and then to compare these results with those obtained by the standard electrometric method. If the difference is sufficiently constant, it can be used as a correction, so that results obtained at room temperature by the simple colorimetric method may be made to indicate the actual pH of the blood plasma. A considerable number of human plasmas have been subjected to pH determination by the two methods, and it now appears probable that the problem may be successfully solved. The colorimetric technique was first developed to a point where duplicate readings could be checked within 0.02 pH. The freshly drawn blood is centrifuged under a layer of solid paraffin to prevent loss of carbon dioxide, and a sample is diluted with carbon-dioxide-free physiological saline solution. The latter is contained under paraffin oil in a tube, and has the indicator, phenol red, already in it before the plasma is added. The reading is made in a comparator. Thus far the results have averaged 0.18 pH higher than the electrometric, and correction does not seem to vary by more than 0.02 or 0.03 pH from this value. If similar results continue to be obtained, they will demonstrate that,

with the colorimetric technique developed by Dr Cullen, reducing the pH obtained by 0.18 gives the actual blood plasma pH with an accuracy hardly less than that of the electrometric determination itself.

Such a method will be of value in the study of disturbances in the acid-base balance involving alterations, not only of alkaline reserve, but of the pH also. These conditions do not usually occur in acidoses of the metabolic diseases, but they do occur constantly in anesthesia, as stated in our last report, and probably in other conditions in which the sensitiveness of the respiratory control is affected.

The above mentioned work on blood changes in anesthesia has been continued, with confirmation of our former results, viz. that etherization causes a lowering of both alkaline reserve and pH in the blood, the respiratory center suffering an apparently progressive deadening from the start of anesthesia to the deepest stage. With the improvements in technique on which we are at present engaged, including the colorimetric pH determination, we hope shortly to learn more about the progress of the changes, their persistance, and factors affecting their severity and the rate of recovery.

We also expect to use this technique to study more completely than heretofore the acid-base balance of the blood in pneumonia; Newburgh, Means and Porter reported evidence that in experimental pneumonia the respiratory center becomes less responsive to carbon dioxide stimulus. If this is the case, a decrease in the pH of the blood is to be expected, similar to that found in anesthesia

Dr Cullen, Dr Austin, and Dr Peters are continuing the work begun by them with Dr McLean, in a collaboration between this laboratory and that of Prof. L. J. Henderson of Harvard, with the purpose of ascertaining the quantitative relationships of the changes that occur during respiration in the carbon dioxide and oxygen, the chloride and bicarbonate, of the cells and plasma. The preliminary results were of sufficient interest to indicate that the problem is worth developing, for the light it may throw on the physiology of respiration, and the additions that it may bring to our means for studying respiratory and circulatory disease. We are at present improving certain points in the technique which were found to need standardization before results of the desired accuracy could be relied upon.

Dr Peters is engaged in the preparation of crystalline hemoglobin for study of its acid dissociation constant and of its alkali-binding power and buffer effect. Hemoglobin appears to be the most important substance in the blood in maintaining its constancy of reaction during the physiological changes of respiration, and an accurate knowledge of the above factors will assist in formulating clearly the manner in which hemoglobin stabilizes reaction.

Dr Binger, for work with heart patients, has so improved the Lundsgaard-Van Slyke technique for determination of lung volume that it can be depended upon to give accurate results with subjects who cannot increase the depth of their respirations, or otherwise collaborate. In the original method a known volume of pure oxygen from a bag was mixed with the air in the subject's lungs by several

deep breaths, and the lung volume was calculated from the extent to which the nitrogen gas of the lung air was diluted by the known volume of oxygen. When applied to patients who could not breathe deeply, and therefore require 2 or 3 minutes, instead of 30 seconds, for complete mixture of the gases, the above method becomes inaccurate because of the considerable volume of oxygen absorbed. This difficulty has been overcome by having the subject breathe a mixture of oxygen and hydrogen, and basing the calculation, not on the nitrogen content of the final gas mixture, but on the N_2:H_2 ratio. Since hydrogen is absorbed but slightly, accurate results may be obtained by this method even when the breathing is continued for several minutes.

The object of this work is to analyze the lung volume changes in heart patients. Peabody has already shown that the vital capacity is diminished, roughly in proportion to the cardiac insufficiency. Dr Binger's object is to obtain data also on the residual air, and find whether the diminished vital capacity is due to inability to expire with normal completeness, or to the obliteration of part of the aerating lung volume. Results of interest are being obtained, and will be reported later.

Dr Stadie is continuing the studies begun last year of the effect of oxygen breathing in pneumonia. He has improved the structure of the chamber in which the patients are placed, and perfected the methods for removing the carbon dioxide and controlling the oxygen tension. His results confirm those obtained last year in showing that cyanotic patients have the oxygen content of their blood increased by raising the oxygen content of the air they breathe, that

the cyanosis correspondingly diminishes, and usually disappears, the respiration and pulse become slower and more normal, and the patients appear more comfortable. The improvement lasts only as long as oxygen administration continues, removal from the chamber resulting in an almost immediate return to the former condition. The course of the infection appears not to be affected. The treatment may, however, have value in maintaining the vitality of patients who have the ability to overcome the infection if physical exhaustion is prevented.

Dr Stadie is beginning a study of the changes in effective lung volume during convalescence from pneumonia, particularly with a view to obtain information concerning the changes that accompany resolution. For this purpose he is employing the improved technique for lung volume determination perfected by Dr Binger.

Dr Edgar Stillman is continuing the study of nephritis, devoting particular attention to the relation of the rates of salt and urea excretion to the blood concentration, and to other functional tests in the different types of nephritics. At the same time the treatment is accurately regulated with the aid of the diet kitchen. It is hoped that information may gradually accumulate which will assist in the objective differentiation of the types of nephritics and the corresponding treatments which are necessary and justified.

Dr Stillman, as one of the details of his study, has, at Dr Cohn's suggestion, made observations of the blood volume by the vital red method in a series of cases with hypertension, in order to find whether there is any indication of a decrease in the volume of the arterial system. The results were entirely normal, however.

Miss Hiller has completed a careful study of the different protein precipitants used for blood, in preparation for further experiments on amino acid physiology which will require especial accuracy in removal of the proteins. The work has indicated the relative completeness with which the different precipitants remove both the native proteins and the intermediate albumoses and peptones, and will serve as a guide in the use of reagents that could formerly be employed only in a relatively uncertain manner.

Miss Hiller is alos proceeding with the very difficult work of isolating and identifying the unknown basic amino-acid of gelatin. The substance has been prepared in small quantities in form for analysis, and is now being prepared on a larger scale.

Dr Cohn

During the past six months, the laboratories in the Central Building (Room 320) and on the sixth floor of the hospital have been abandoned, and the new one on the eighth floor occupied in their stead. Under ordinary circumstances this event would merit no comment, but the planning and preparation of the new rooms and the installation of apparatus consumed so much of time and of energy as seriously to interfere with the prosecution of work. The loss is, however, not to be taken seriously, for the new laboratories offer so much greater facilities, conserve our energies so much more and render experimenting so much easier as to compensate fully for the work spent in their construction.

Investigations have been carried on, both on patients and on animals. (1) Patients in Dr Swift's care, suffering from acute

rheumatic fever, have been studied electrocardiographically. Changes of two sorts have taken place in the hearts of these patients. The first concerns alterations in the auriculo-ventricular conduction interval. It is generally presumed that in health this is constant or changes within narrow limits. But it is not unknown that in this disease, as in syphilis, changes take place in A-V conduction in certain instances, which sometimes lead to heart block, especially as a late sequela. But this knowledge did not lead us to expect changes from day to day, amounting from 0.02 to 0.05 seconds. This occurrence furnishes sound clinical evidence for the belief that the rheumatic process may involve the heart in its entirety, myocardium as well as endocardium, and in a sense permits studying the myocardial involvement in detail. Exactly what lesion it is, which is responsible, one cannot say, but the nodules described by Aschoff as characteristic of the disease suggest themselves as the cause of this manifestation. It is interesting to notice that cases in which other evidence of continuing disease has ceased to exist, still manifest the changes which are now described.

A second point which this study has shown is alteration in the form of the ventricular complexes in brief periods of time. This involves more especially the Q R S group and is indicated in the changing height of the waves; alterations in the notchings when these are present; and deviations in the form of that portion of the curve lying between the Q R S group and the T-wave. In one case especially was this change marked. The downstroke of the R-wave failed to reach the base line, but stopped short of this and continued into

the T-wave. It is for the moment of importance to recognize these changes in the curves. That they betoken the presence of alterations in the heart muscle is, of course, true. But which function in the heart has suffered and for what reason is still obscure. The full explanation must await a closer knowledge of the electro-physiology of the heart.

In the third place, irregularities have been encountered in these cases. Premature contractions, especially of the ventricular variety, are not infrequent. In two instances transient fibrillation of the auricles was seen. In one case several paroxysms, two or three, were studied. In the other, a single paroxysm was observed, but before the return to normal mechanism, there was an interval in which for two or three days the auricles fluttered. We were in this case uncommonly fortunate in being able to photograph the transition from fibrillation of the auricles to the normal mechanism. I believe that in human physiology this observation is unique and important. In the curve after the last ventricular wave occurring during the fibrillatory period, several more auricular waves indicative of fibrillation appear. Then all evidence of auricular activity vanishes. This post fibrillatory diastolic period is distinctly longer than that usual in this case. Diastole is interrupted by a P-wave, representative of coordinate auricular contraction. It is followed by waves of normal ventricular activity and thereafter by a normal mechanism. The curve throws light on the fibrillatory process. Whatever its nature, whether due to multiple foci of stimulus production as Lewis holds, or to circus

contractions according to Garrey and Mines, the process obviously terminates suddenly. That this is less likely on Lewis' supposition than on that of Garrey and Mines is possible. For the moment further speculation is perhaps unwarranted.

(2) Further studies have been made with the dogs prepared after the manner of Van Leersum. In experiments performed on anesthetized dogs, it was found that with doses of g-strophanthin and of digitalis equivalent to therapeutic doses, increase of contraction, increase of blood pressure, and change in the T-wave all took place. Whether these changes take place in the intact, unanesthetized dogs is of course unknown. It was thought that the dogs prepared after the Van Leersum method offered an opportunity to make this test. They afforded at all events an opportunity to study the blood pressure and the T-wave change simultaneously. I am unable to report on the results of these experiments. The perfection of the technique for taking the blood pressure has consumed much time. The original method of circular compression of the carotid tunnel had to be abandoned in favor of lateral compression. The training of the dogs to lie still for the duration of the experiment, while not too great a trouble, has nevertheless been difficult. Meanwhile it is possible to report that the collection of control electrocardiograms and of blood pressure readings is being accomplished.

(3) Studies which were made with Dr Noguchi on monkeys and guinea pigs inoculated with leptospira icteroides and leptospira icterohemorrhagiae have been completed. As in the case of yellow fever in human beings, the heart rate in experimental animals is

slow, out of proportion to the temperature elevation. The mechanism of slowing is one of the whole heart, that is to say, it involves the sinus rhythm. Irregularities were encountered but they were not significant. Animals in which typical disease did not develop and which were killed, did not react as the infected ones did; the heart rate did not become slow but remained at the normal level.

Dr Levy has studied the size of the heart in pneumonia. In a group of pneumonia patients serial teleroentgenograms were made during the acute stages of the disease and in convalescence. Linear measurements of the cardiac diameters and planimetric determinations of the size of the heart's silhouette have shown that in a large percentage of cases the heart dilates during the febrile period. The return to its normal size is gradual, lagging behind the decline of the pulse and temperature curves. In a series of control cases, both normal and pathologic, no significant alteration in the size of the heart has been found over relatively long periods of time.

For outlining the cardiac silhouette a new technique has been devised which substitutes exact instrumental delineation for the older free-hand method of completing the outline at the base and at the diaphragm. From the x-ray plate four points and two arcs, at the right auricle and left ventricle, may be directly traced. The cardiac outline is completed by describing the two remaining arcs with the compass, employing as radius the distance between the two points which it is desired to connect. In this fashion the sector outlined by the arc joining these two points is a function of the distance between the two points which are joined, and its area varies

therefore, directly with this distance. By this method the total actual shadow cast by the heart on the plate is not the area measured, but a definite and constant portion of it.

2. We have continued the observations begun last year on the use of strophanthin in the treatment of heart failure. A group of patients suffering from fibrillation of the auricles has received the drug intravenously. In addition to the usual clinical methods employed, a study of the venous pressure was made. It has been found that the venous pressure rises as heart failure develops, falling rapidly with improvement in the clinical condition. The venous pressure in normal individuals in bed ranges from about 5 to 15 cm. of water; in heart failure it is not uncommon to find the venous pressure rise to 25 cm. of water. After treatment with strophanthin, in as short a period as one hour, the venous pressure has fallen from this level to 5 cm. of water with coincident relief in the patient's symptoms. This study is being continued.

3. The same group of patients has at different times received digitalis by mouth. Dosage and the rate of utilization of the drug in the body have been particularly studied. From the observations of Eggleston it has become clear that large doses of digitalis may be given by mouth safely and with therapeutic effects in surprisingly short time. Our own studies have confirmed these findings. It appears, however, that to attempt to calculate the dose of digitalis by the body weight method, as Eggleston recommended, is disappointing.

The difficulty appears when one considers that the optimum therapeutic effect, which may be determined easily by available criteria, is obtained in the same patient on different occasions by doses of

different magnitude and is observed in different patients of the same weight after quite different amounts of the drug. Other methods for arriving at the proper dosage have been suggested and studied. The amount of the muscle of the heart has, for example, been supposed to bear a relation to the amount of the striated muscle mass in the body in the belief that striated muscle is the most active tissue which the heart is called upon to supply. To estimate this mass creatinin determinations in the urine have been made, for the excretion of creatinin is constant and is dependent on the volume of striated muscle. Here again there has been disappointment, for the amount of creatinin bears no constant relation to the effective dose of digitalis. The same may be said of the use of the surface area in relation to digitalis dosage. The attempt to utilize an estimate of the rate of excretion or utilization of digitalis over a long period of time is also unavailing in patients who require continued digitalization. In brief, there are many unknown factors concerned in the mechanism of heart failure, so that it is unsound to attempt to calculate the dosage of digitalis in terms of any one of them, or so far as we know now on the basis of any combination. The dose to be given to a given patient at any time or to the same patient at different times must apparently still be estimated according to criteria we have so far found useful - the fall in rate in auricular fibrillation; the change in the T-wave of the electrocardiogram; obvious changes in breathing; and the subjective sensations of the patient.

4. A <u>new strophanthin compound</u>, obtained from Dr Jacobs and Dr Heidelberger of the Rockefeller Institute, has been experimented

with by Dr Levy in cats. It is obtained from g- strophanthin by hydrolysis, and from the preliminary observations which have been made in animals, appears to be less toxic, though therapeutically as effective as the mother substance. Provided this compound can be isolated in crystalline form by the chemists, further studies, eventually to include observations on patients, will be made.

Dr Binger has devised a method for studying accurately the lung volume in individuals who are short of breath. This method was reported at a recent meeting of The Society for Experimental Medicine and Biology. At present this method is being applied to cases of compensated heart disease and to cases of heart failure in various stages of decompensation. Cases of auricular fibrillation lend themselves admirably to this study, for the degree of decompensation can be controlled by digitalis therapy. As effort is being made to correlate certain symptoms and physical signs with lung volume changes both absolute and percentile. Data are being accumulated but are not yet sufficient to serve as the basis of generalization which it is hoped will throw light on the mechanism of reduced vital capacity and of dyspnea.

The problem of dyspnea is being studied also from the point of view of circulatory stagnation.

Other phenomena of circulatory stagnation are being investigated in patients and in animals. Of particular interest appear to be: (1) The relation of oxygen content and capacity of arterial and venous blood. It has been found that content and capacity bear an inverse relationship in animals in experimentally produced anoxemia.

A similar study will, it is hoped, be undertaken to examine this relationship in patients under conditions of anoxemia seen in heart disease. (2) The relation of elevation of temperature to heart failure and circulatory stagnation. An hypothesis has been found which appears to explain this relationship. It is that the elevation of rectal temperature which has been found results from faulty heat dissipation. Data on body temperature are being accumulated in a preliminary way in a variety of pathologic states. The facts so far obtained appear to throw light on the fever seen in heart failure and to associate its occurrence with circulatory stagnation.

Dr Raisbeck has continued in the manner in which, as has been reported from time to time, he began to make measurements of electrocardiograms made here of soldiers returned from France. It is, as has been pointed out, a laborious undertaking and will require some time to complete. His work is admirable and leads to the expectation that its results will serve as the basis of reliable generalizations on the dimensions of the normal electrocardiogram.

Recently we have administered quinidin sulphate, a cinchona derivative, to patients. Formerly quinin was much used as a cardiac remedy. Recently (1918) quinidin, a stereoisomer of quinin, was introduced by Frey in Schittenhelm's clinic. He found it effective in converting auricular fibrillation into a normal cardiac mechanism in a routine investigation of the usefulness of drugs of this series. Since his original publication, the reports of 83 cases of auricular fibrillation, in which the drug has been used, have been published. In 42, or 50.6 per cent of these cases, the fibrillatory mechanism of

the heart is reported to have been successfully restored to a normal sinus rhythm.

This effect was so astonishing that Dr Levy was induced to repeat the German observations. In the past few weeks the drug has been administered to four patients suffering from this condition. All these patients were known to be subject to fibrillation for long periods of time. In two patients success in restoring the normal cardiac mechanism was actually attained. In a third, transient auricular flutter followed the administration of the drug; and in the fourth case no effect was attained. It is possible that with increasing knowledge a greater percentage of success is possible, especially in cases like the ones in which auricular flutter occurred. The duration of the effect of this drug, if one may judge from the experience cited in the literature, is short, lasting on the average one week to one month; the longest case observed remained normal four months. The first patient observed here has now maintained a normal mechanism for ten days. We are studying the drug, both in patients and in experiments.